REMARKS

Claims 1-13 and 17-19 are pending. The Applicant herein respectfully requests further examination of the application and reconsideration of the claims, in view of the amendments and remarks presented herein.

Claim 1 has been amended to require "prepared for administration" to limit the scope to of the claim to compounds that have been prepared in a substantially isolated form for pharmacological formulation and administration to a patient.

Rejections under 35 USC §112, pargraph 1

I. The subject matter of each of claims 13 and 17-19 of the pending application is alleged by the Examiner to lack support by the disclosure under the statutory requirement for written description. The Examiner particularly alleges that "the instant specification does not adequately describe the nexus between the modulation of at least one nuclear receptor and a usefully treatment of a disease condition."

Independent claim 13 is now amended to specify Peroxisome Proliferator-Activated Receptor (PPAR) as the required nuclear receptor.

The Applicants respectfully point out that Peroxisome Proliferator-Activated Receptors (PPAR), including PPARγ and PPARδ, for example, are ligand-activated transcriptional factors that belong to the nuclear hormone receptor superfamily, which are essential in controlling lipid, glucose, and energy homeostasis.[1] PPAR are in fact well-known in the art to mediate type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example. As highlighted in the Background of the Applicants' invention, modulation of the biological activity of these receptors is indeed widely-accepted in clinic practice and is well-documented in many preclinical and clinical studies for controlling and treating associated disease conditions including but not limited to human type 2 diabetes and dyslipidemia.[2] The PPARγagonists Rosiglitazone and

[1] Issemann, I.; Green, S. Nature 1990, 347, 645; Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B.R. J. Med. Chem. 2000, 43, 527.

[2] The Examiner is respectfully referred to the subject Specification, for example, at page 2, line 18 to page 4, line 14.

Piglitazone, for example, are well-known for treating human type 2 diabetes. PPARα agonists Fenofibrate and Clofibrate, for example, are well-documented for treating human dyslipidemia.

The association of the modulation of the biological activity of PPAR receptors in treating disorders is indeed historically enriched by many years scientific research that reveals PPAR mechanistic action. In short, PPARs function as transcriptional factors by forming heterodimers with their partner RXR (retinoid X receptor) to bind to the upstream region of a PPAR-targeted gene, leading to the activation of the gene expression by cognate PPAR agonist.

It is well documented that several groups of genes controlling glucose and lipid homeostasis in humans are controlled by PPAR activation, in which three subtype of PPAR, namely PPARα, PPARδ, and PPARγ selectively activated these genes according to our body's metabolic condition in physiological state.

It is also well reported that agonists of PPAR are generally effective to treat and control disease conditions in animal models -- further indicated for clinic efficacy -- in treating and controlling diseases recited by the Applicants in the disclosure.

It is these historic scientific studies that have well- established the link between activation of one of PPARs and their application in preventing, controlling, and treating type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example.

The Applicants enclose herewith an example publication entitled Design, synthesis, and evaluation of a new class of noncyclic 1,3-dicarbonyl compounds as PPARα selective activators,[3] which moreover, in the introduction (see footnotes), for example, illustrates the state of the art at the time of the Applicants' invention.

A well-established and routine art-accepted protocol to evaluate a candidate agent's agonistic activity of PPAR is by means of a cell-based reporter gene assay. Particularly, activation of PPAR in cell-based reporter gene assays is an art-accepted model that corresponds very closely to efficacy of the agent *in vivo*. The Applicants indeed employed such a technology (reporter gene assay) and, in doing so, unambiguously exemplified species representative of the invention, *see* Figures 1, 2, 5, 6, 7, 8, 9, and 10, for example. The representative species are illustrated to exhibit agonistic activity of PPAR with characteristics of distinct activation profile

[3] Bioorganic & Medicinal Chemistry Letters 14 (2004) 3507–3511.

over PPARγ, or PPARγ and PPARα, or PPARα, γ, and δ.[4] Representative species are further tested, as disclosed, in relevant animal model of *db/db*, a well-established and commonly known transgenic model which mimics late stage pathological changes in human type 2 diabetes. Efficacy is illustrated in treating various disease conditions as exemplified in Figures 3 and 4.

In summary, one skilled in the art, in view of the state of the art - *and* - the facts of the Applicants' Specification, can easily recognize the disclosure and identification of agonist compounds of the present invention as well as their art-expected use in preventing, controlling, and treating type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example.

Accordingly, the applicants respectfully request the Examiner to withdraw the rejection.

II. The subject matter of claims 13, and 17-19 of the pending application is alleged by the Examiner to lack support by the disclosure under the statutory requirement for enablement.

The statute requires that the Applicant must teach one of ordinary skill in the art how to make and use the invention, as claimed, in view of what is known in the art.[5]

Independent claim 13 is now amended to specify Peroxisome Proliferator-Activated Receptor (PPAR) as the required nuclear receptor.

As stated *supra*, the art is very well-developed, i.e., PPAR are in fact well-known in the art to mediate type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example. Modulation of the biological activity of these receptors is indeed widely-accepted in clinic practice and is well-documented in many preclinical and clinical studies for controlling and treating associated disease conditions including but not limited to human type 2 diabetes and dyslipidemia. The PPARγagonists Rosiglitazone and Piglitazone, for example, are well-known

[4] Reflected by the fold activation over the basal level and comparing to positive control such as Ros for PPARγ, Wy for PPARα, and 2-Bro for PPARδ.

[5] If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC§112 ¶1 is satisfied. MPEP §2164.01(c). For example, it is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation. If one skilled in the art, based on knowledge of compounds having similar physiological or biological activity, would be able to discern an appropriate dosage or method of use without undue experimentation, this would be sufficient to satisfy 35 USC§112 ¶1.

for treating human type 2 diabetes. PPARα agonists Fenofibrate and Clofibrate, for example, are well-documented for treating human dyslipidemia.

As stated *supra*, the Applicants unambiguously exemplified species representative of the invention, *see* Figures 1, 2, 5, 6, 7, 8, 9, and 10, for example, illustrated to exhibit agonistic activity of PPAR with characteristics of distinct activation profile over PPARγ, or PPARγ and PPARα, or PPARα, γ, and δ.[6]

Figure 1 graphically illustrates comparative activation of RXR/PPAR alpha heterodimers by compounds of the present invention (Example 30).

Figure 2 shows comparative activation of RXR/PPAR gamma heterodimers by compounds of the present invention (Example 31).

Figure 5 shows comparative activation of RXR/PPAR alpha heterodimers by compounds of the present invention (Example 34).

Figure 6 shows comparative activation of RXR/PPAR gamma heterodimers by compounds of the present invention (Example 34).

Figure 7 shows comparative activation of RXR/PPAR delta heterodimers by compounds of the present invention (Example 34).

Figure 8 graphically illustrates comparative activation of RXR/PPAR alpha heterodimers by compounds of the present invention (Example 35).

Figure 9 graphically illustrates comparative activation of RXR/PPAR gamma heterodimers by compounds of the present invention (Example 35).

Figure 10 graphically illustrates comparative activation of RXR/PPAR delta heterodimers by compounds of the present invention (Example 35).

Representative species are further exhibited as efficacious in relevant animal model of *db/db*, a well-established and commonly known transgenic model which mimics late stage pathological changes in human type 2 diabetes. Efficacy is illustrated in treating various disease conditions as exemplified in Figures 3 and 4.

Figure 3 graphically illustrates *in vivo* blood glucose lowering effected by a compound of the present invention (Example 32).

[6] Reflected by the fold activation over the basal level and comparing to positive control such as Ros for PPARγ, Wy for PPARα, and 2-Bro for PPARδ.

Figure 4 graphically illustrates *in vivo* blood triglyceride level lowering effected by a compound of the present invention (Example 33).

Further, toward the issue of enablement, the Applicants also enclose herewith an example publication entitled Design, synthesis, and evaluation of a new class of noncyclic 1,3-dicarbonyl compounds as PPARα selective activators,[7] which illustrates *in vitro* and *in vivo* results of PPAR selective activators within the scope of the instant claims that are useful against type 2 diabetes, for example, as well as other metabolic diseases.

The Applicants respectfully point out that *some* experimentation by one of ordinary skill to practice the invention as claimed is acceptable under 35 USC §112.1, so long as it is not undue. As a corollary, the Applicants respectfully submit that one of skill in the art can readily practice the invention as claimed in view of the current state of the art -- without undue experimentation, particularly since efficacious compounds that similarly modulate Peroxisome Proliferator-Activated Receptors (PPAR) are well-known in the art.

The Applicants respectfully request the Examiner to withdraw the rejection.

III. The subject matter of claims 1-13, and 17-19 of the pending application is alleged by the Examiner to lack support by the disclosure under the statutory requirement for enablement. The Examiner's position at this time is that the specification fails to provide sufficient support of the genus of compounds or the use of the compounds of claim 1, for example.

The Applicants respectfully point out to the Examiner that it is commonly understood from medicinal chemistry point of view that a subtle change in chemical structure in receptor-ligand interaction can dramatically change the respective biological activity. Accordingly, Fig 7 merely indicates that compounds 1200205 (methoxycarbonyl) and 1200207 (carbamoyl) exhibit a large difference in agonist activities of RXR/PPARδ having activities respectively of 3.75 and 2.0 -- for a small difference in structure. Rich references indeed exist in the literature along these lines and the Applicants profess that similar examples can be provided if the Examiner so desires.

Selected species exemplified in the instant Specification illustrate various activation of different PPAR subtypes. For example, several species of structure were selected as shown in

[7] Bioorganic & Medicinal Chemistry Letters 14 (2004) 3507–3511.

Example 13 (Compound CS0130012), Example 14 (Compound CS0130013), Example 15 (Compound CS0130014), Example 20 (Lab code CS01200204), Example 21 (Lab code CS01200205), Example 23 (Lab code CS01200207), Example 26 (Lab code CS01200304), Example 27 (Lab code CS01200305), and Example 29 (Lab code CS01200307) that minor structural changes result in altered activation profile over PPARα, PPARγ, and PPARδ individually as exemplified in Fig 1, 2, 5, 6, 7, 8, 9, and 10 (Example 30, 31, 34 and 35). Strong structure/activity relationships exist for the genus of the claim 1. Such a structure activity relationship is predicable by one of ordinary skill in the art in view of the disclosure and what is known in the art. Provided herein, for example, is a published article by the Applicants related to structures within the scope of claim 1. A strong structure activity relationship is reported -- which can also be identified in the subject disclosure. However, the Applicants respectfully remind the Examiner that enablement of an invention at the time of filing -- may be established by evidence generated at a later point in time. Nevertheless, it is within the skill of an ordinary medicinal chemist to predict, to a reasonable extent, what structure change will alter a ligand's activity against a specific receptor, in this case PPAR, for example., in view of the examples provided in the Applicants' Specification. The Applicants moreover submit that it is within the skill of an ordinary medicinal chemist to predict, to a reasonable extent without undue experimentation, species having PPAR agonist activity as exemplified that are prospectively useful in treating and controlling type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example.

In summary, the current application unambiguously teaches representative species having PPAR agonist activities within the proper scope of claim 1 that are useful in controlling type 2 diabetes, dyslipidimia, cardiovascular disease, and syndrome X, for example. PPARs play a critical role in mediating pathological conditions of the diseases as documented by rich literature and clinical studies. The selected representative examples are fundamentally serial new chemical entities having PPAR agonist activities *in vivo* with well-defined structures defined by claim 1. Although some experimentation may be required to practice the full-scope of the subject matter of the claim, undue experimentation is certainly not required to evaluate which disease conditions, for example, can be treated by the compounds of claim 1 with a success rate

recognized as acceptable by the scientific community -- in view of the disclosure *and* the state of the art.

The Applicants respectfully request the Examiner to withdraw the rejection.

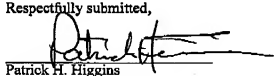
The Applicants respectfully request the Examiner to withdraw all rejections under 35 USC §112, pargraph 1.

* * *

For the foregoing reasons, the Applicant submits that Claims 1-13 and 17-19 are in condition for allowance. Early action toward this end is courteously solicited.

The Commissioner is authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-1943.

Respectfully submitted,



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DATE: September 22, 2005

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► *The following report is attached hereto*:

Design, synthesis, and evaluation of a new class of noncyclic 1,3-dicarbonyl compounds as PPAR selective activators, Bioorganic & Medicinal Chemistry Letters 14 (2004) 3507–3511.

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Design, synthesis, and evaluation of a new class of noncyclic 1,3-dicarbonyl compounds as PPARα selective activators

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Abstract—Lipid accumulation in nonadipose tissues is increasingly linked to the development of type 2 diabetes in obese individuals. We report here the design, synthesis, and evaluation of a series of novel PPARα selective activators containing 1,3-dicarbonyl moieties. Structure–activity relationship studies led to the identification of PPARα selective activators (compounds 10, 14, 17, 18, and 21) with stronger potency and efficacy to activate PPARα over PPARγ and PPARδ. Experiments in vivo showed that compounds 10, 14, and 17 had blood glucose lowering effect in diabetic db/db mouse model after two weeks oral dosing. The data strongly support further testing of these lead compounds in other relevant disease animal models to evaluate their potential therapeutic benefits.

1. Introduction

Increasing evidence suggests that lipid accumulation in nonadipose tissues, such as pancreatic islet cells and skeletal muscle, is causally related to the development of type 2 diabetes in obese individuals.[1] Peroxisome proliferator activated receptor-α (PPARα), PPARγ and PPARδ are ligand-activated transcriptional factors that belong to the nuclear hormone receptor superfamily, which are essential in controlling lipid, glucose, and energy homeostasis.[2,3] At present, PPARγ agonists rosiglitazone[4] (1, Fig. 1) has been successfully prescribed for patients having type 2 diabetes. The fibrate agents, such as fenofibrate and Wy-14643[5] (2), are low affinity PPARα agonists; fenofibrate and beazfibrate have been widely used clinically as antihyperlipidemic drugs. These drugs increase HDL cholesterol levels and lower LDL and VLDL cholesterol levels with stronger triglyceride-lowering effect than statins, the HMG-CoA reductase inhibitors. Recently published data showed that PPARδ activators could also be used in the treatment of

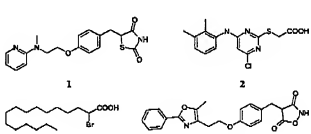

Figure 1. Reference compounds.

Keywords: PPARα activators; Type 2 diabetes; Noncyclic 1,3-dicarbonyl compounds; Metabolic diseases.
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dyslipidemia.[5] Fatty acids, for example, 2-bromo-hexadecanoic acid[6,7] (3) are known as weak activators of PPARδ. Because of the side effects of PPARγ agonists, such as weight gain and water retention,[8] and the potential beneficial effects of the PPARα and PPARδ,[9] balanced triple agonists for PPARα/γ/δ or selective agonists for PPARα or δ are of great interest for drug discovery efforts worldwide.[10]

Here, we describe the design, synthesis, and evaluation of a new class of PPARα selective activators containing 1,3-dicarbonyl moieties, leading to the identification of PPARα selective activators as candidate leads for the potential treatment of metabolic disorders such as diabetes, obesity, and hyperlipidemia.

2. Result and discussion

JTT-501[11] (4), a dual agonist of PPARα/γ, is an isoxazolidine-3,5-dione. Its activity is likely to be mediated through a malonic amide metabolite that is generated by hydrolysis of the heterocyclic ring.[12] We therefore took this moiety as template to develop structurally new human PPARα selective activators.

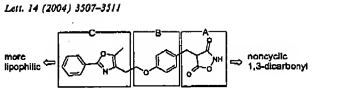
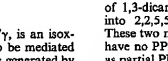


Figure 2. Chemical structure of the lead compound JTT-501.

The framework of JTT-501, like most of PPAR activators, can be divided into three key regions: (A) the acidic head part, (B) the linker part, and (C) the hydrophobic tail part (Fig. 2). Thus, chemical modifications of the acidic head part and the lipophilic part[13] might change their selectivity to PPARα, PPARγ, or PPARδ receptors.

We first modified the acidic head part into a series of 1,3-dicarbonyl compounds, and the lipophilic part into 2,2,5,5-tetramethyl-2,3,4,5-tetrahydronaphthalene. These two modifications generated compounds 5–9 that have no PPARα and PPARδ activities while remaining as partial PPARγ agonists (Table 1). Substitution of the four methyl groups with hydrogen atoms from this lipophilic tail formed compounds 10–13 that had activities to all three PPAR subtypes, suggesting that

Table 1. Cell-based in vitro transcription activation of test compounds on hPPARα, hPPARγ, and hPPARδ[a]



No.	R1	R2	R3	PPARα EC_{50} μM (%max[b])	PPARγ EC_{50} μM (%max[b])	PPARδ EC_{50} μM (%max[b])
5	$-COOCH_3$	$-COOCH_3$	2,2,5,5-Tetramethylcyclohexano	ia[c] (–)	0.15 (66)	ia (–)
6	$-COOCH_3$	–COOH	2,2,5,5-Tetramethylcyclohexano	ia (–)	0.09 (66)	ia (–)
7[d]	–COOH	–COOH	2,2,5,5-Tetramethylcyclohexano	ia (–)	2.05 (9)	ia (–)
8	$-COOCH_3$	$-CONH_2$	2,2,5,5-Tetramethylcyclohexano	ia (–)	0.83 (15)	ia (–)
9	–COOH	$-CONH_2$	2,2,5,5-Tetramethylcyclohexano	ia (–)	ia (–)	ia (–)
10	$-COOCH_3$	$-COOCH_3$	Cyclohexano	0.03 (210)	1.5 (53)	3.31 (134)
11	$-COOCH_3$	–COOH	Cyclohexano	ia (–)	0.69 (60)	2.45 (100)
12[d]	–COOH	–COOH	Cyclohexano	ia (–)	2.11 (39)	4.68 (80)
13	$-COOCH_3$	$-CONH_2$	Cyclohexano	ia (–)	3.43 (22)	ia (–)
14	$-COOCH_3$	$-COOCH_3$	Benzeno	0.07 (264)	0.53 (78)	1.27 (144)
15[d]	–COOH	–COOH	Benzeno	ia (–)	ia (–)	ia (–)
16	$-COOCH_3$	–COOH	Benzeno	0.044 (264)	1.12 (59)	1.05 (125)
17	–COOH	$-CONH_2$	Benzeno	0.25 (213)	3.02 (8)	2.63 (111)
18	$-COOCH_3$	$-COOCH_3$	Hexahydropyridino	0.17 (215)	3.16 (27)	3.16 (67)
19	$-COOCH_3$	–COOH	Hexahydropyridino	0.09 (207)	3.80 (14)	3.09 (56)
20[d]	–COOH	–COOH	Hexahydropyridino	ia (–)	ia (–)	ia (–)
21	–COOH	$-CONH_2$	Hexahydropyridino	0.14 (210)	2.88 (14)	3.62 (83)
1	Rosiglitazone			7.3 (16)	0.04 (100)	9.3 (42)
2	Wy-14643			15.5 (100)	ia (–)	1.46 (20)
3	2-Bro			nd[e]	nd	3.72 (100)
4	JTT-501[f]			1.9 (NA)	0.083 (NA)	ia

[a] Data represent the mean values from at least three independent experiments each in triplicate.
[b] %max responses were calculated as Wy-14643 at 5 μM as 100% for PPARα; Rosiglitazone at 1 μM as 100% for PPARγ; and 2-bromohexadecanoic acid at 5 μM as 100% for PPARδ.
[c] ia = inactive.
[d] Compounds 7, 12, 15, and 20 are chemically instable and decomposed to CO_2 and monoacid at different rates when activity measured in vitro.
[e] nd = not determined.
[f] The EC_{50} originated from a same type of cell-based transcription activation assay reported for JTT-501 was adapted from reference 3; NA: not available.

the 2,2,5,5-tetramethyl-2,3,4,5-tetrahydronaphthalene group might be too big to interact with PPARα and δ favorably. Changing the lipophilic part to a planar group (naphthalene), such as compounds **14–17**, led to stabilization of the interaction between the compounds and PPARα as evidenced by strong transcription activation of PPARα, although the substitutions were made in the acidic head part of the dimethyl malonate group. The substitution of the dimethyl malonate group in compound **14** with 2-methoxycarbonyl propionic acid (compound **16**) resulted in weakening of PPARγ agonist activity while marginally enhancing PPARα and δ activities; the substitution in compound **14** with 2-carbamoyl propionic acid (compound **17**) caused significant decrease in PPARγ activity, as indicated in Table 1, where the maximal response of the PPARγ activity to the compound was only 8% of that of rosiglitazone, but the activation of PPARα by the compound remained strong. Replacement of a carbon atom with a nitrogen atom in the 2,3,4,5-tetrahydronaphthalene ring in compounds **18–21** further decreased their ability to activate PPARγ and δ, while relatively strong PPARα activation by these compounds still remained. The activity profiles in Table 1 indicate that the compounds **10** and **19** are potent activators with good selectivity for PPARα while the compounds **17**, **18**, and **21** also exhibit good selectivity for PPARα since the efficacies for PPARγ were significantly decreased; the compounds **14** and **16** show only modest selectivity for PPARα.

AutoDock[14–17] was used to dock compound **17** into PPARα co-crystallized with AZ 242,[18] PPARγ co-crystallized with rosiglitazone,[19] and PPARδ co-crystallized with GW2433,[20] respectively. Figure 3 shows that compound **17** docks into the active sites with the carboxylic acid in the same position as the co-crystallized ligands in PPARα and PPARδ, but not in PPARγ. The calculated pK_i by AutoDock were 8.14, 5.89 and 7.06 to PPARα, PPARγ, and PPARδ. This data is in good agreement with the in vitro activities measured.

Compounds **10**, **14**, and **17** were tested in preliminary experiments using diabetic db/db mouse model. As shown in Table 2, all three compounds were able to lower the increased blood glucose levels as compared to the vehicle treatment after 12-days oral dosing, but they were less effective than rosiglitazone. The blood glucose-lowering effect of compound **17** is interesting since it is almost inactive for PPARγ stimulation, suggesting that the glucose-lowering effect may indirectly result from its lipid modulation. It should be noted that the compounds **10** and **14** are pro-drug forms that are converted to the acid form quickly in vivo. It is speculated that these two compounds may have short half-lives that could generate a favorable side-effect profile. Efforts are currently underway to test the efficacy of these compounds in other animal models of type 2 diabetes and dyslipidemia.

Table 2. In vivo anti-diabetes activity of tested compounds in db/db mice

Compound	Dosage (mg/kg)	Reduction in BG (%) after two weeks of oral dosing[a]
Vehicle	0	0
10	30	38.15
14	10	35.08
14	30	46.76
17	20	42.22
Rosiglitazone	5	62.96

[a] Data represent the mean of the percent reduction in blood glucose (BG) level measured using blood samples from mice ($N = 4$ to 6 mice).

In conclusion, the in vitro and in vivo results show that by modifying the acidic head part and the lipophilic part of the template compound JTT-501, we obtained PPARα selective activators that are potentially useful for the development of drugs against type 2 diabetic or other metabolic diseases.

3. Experimental Section

3.1. Chemistry

Compounds **5–21** were synthesized as outlined in Scheme 1. The commercially available phenols upon reaction with *p*-bromoethoxy benzaldehyde gave benzaldehyde derivatives **a**. Knoevenagel condensation between the aldehydes **a** and dimethyl malonate gave the benzylidene **b**. Catalytic hydrogenation of **b** with 5% palladium on carbon gave the dimethyl malonate **c**. Partial hydrolysis of **c** with 1 equiv of sodium hydroxide gave the half-ester **d**. The Schotten–Baumann reaction

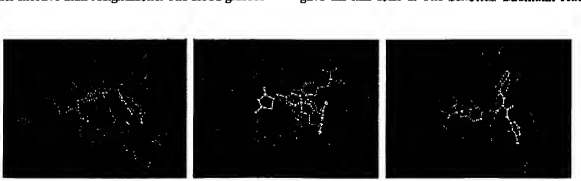
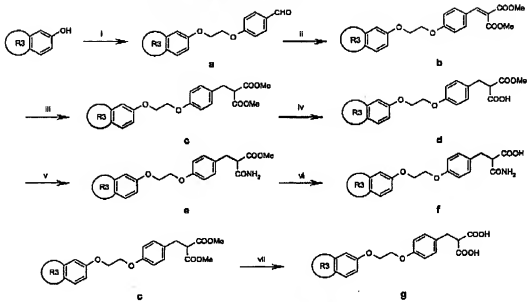


Figure 3. From left panel to right panel: compound **17** (purple) docked into the PPARα receptor co-crystallized with AZ 242 (green), the PPARγ receptor co-crystallized with rosiglitazone (green) and the PPARδ receptor co-crystallized with GW 2433 (green).



Scheme 1. (i) *p*-Bromoethoxy benzaldehyde, KOH, EtOH, refluxing; (ii) dimethyl malonate, toluene, piperidinium acetate, refluxing; (iii) 5% Pd/C, H_2, rt; (iv) 1 equiv of NaOH, rt; (v) $SOCl_2$, 28% ammonia; (vi) NaOH, CH_3OH, rt; (vii) 2 equiv of NaOH, rt.

between an acid chloride of **d** and ammonia gave the amide ester **e**. Hydrolysis of **e** with sodium hydroxide gave the amide acid **f**. Hydrolysis of **c** with more than 2 equiv of sodium hydroxide gave the malonic acid **g**.

3.2. Modeling

The docking program used in this study was AutoDock 3.0.[14-17] Polar hydrogens were added and Kollman partial atomic charges were assigned to the macromolecule. Gasteiger charges were assigned to the ligands. All molecular modeling works except docking were done in Sybyl 6.81 package.[21]

3.3. In vitro transactivation

cDNAs for Human RXR, PPAR were obtained by RT-PCR from the human liver or adipose tissues. Amplified cDNAs were cloned into pcDNA3.1 expression vector and the inserts were confirmed by sequencing. U2OS cells were cultured in McCoy's 5A with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. Cells were seeded in 96-well plates the day before transfection to give a confluence of 50–80% at transfection. A total of 60 ng of DNA containing 10 ng of hRXR, 10 ng of pCMV Gal, 10 ng of nuclear receptor expression vectors and 30 ng of the corresponding reporters were cotransfected per well using FuGene6 transfection reagent according to the manufacturer's instructions. Following 24 h after transfection, cells were incubated with 10% charcoal-stripped FBS DMEM and were treated with the individual compound dissolved in DMSO. The final concentration of DMSO in culture medium was 0.1%.

Cells were treated with compound for 24 h, and then collected with Cell Culture Lysis buffer. Luciferase activity was monitored using the luciferase assay kit according to the manufacturers instructions. Light emission was read in a Labsystems Ascent Fluoroskan reader. To measure galactosidase activity to normalize the luciferase data, 50 μL of supernatant from each transfection lysate was transferred to a new microplate. Galactosidase assays were performed in the microwell plates using a kit from Promega and read in a microplate reader.

3.4. In vivo animal study

Six-week old male db/db mice purchased from Jackson Lab (USA) were given a standard diet and kept at 12 h light/darkness cycle, a temperature of 21 °C, and a relative humidity of 50% throughout the accommodation (two weeks) and dosing periods. All tested compounds were suspended in water containing 0.2% of methylcellulose and 0.1% Tween-80 (w/v). Mice were given tested compounds by gavage once daily for 12 days. Blood was collected from tail vein after 3 h fasting in the morning for blood glucose measurement using Roche's Accu-Chek advantage II Glucose strip together with Accu-Chek Advantage glucose meter.

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